PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
SOPC/P28436PC		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB 03/02459	06.06.2003	07.06.2002

International Patent Classification (IPC) or both national classification and IPC
C12N15/10

Applicant
SOPHION BIOSCIENCE A/S et al.

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
05.01.2004	28.10.2004

Name and mailing address of the international preliminary examining authority:

European Patent Office - P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk - Pays Bas
Tel. +31 70 340 - 2040 Tx: 31 651 epo nl
Fax: +31 70 340 - 3016

Authorized Officer

Hornig, H

Telephone No. +31 70 340-2620

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB 03/02459

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-26 as originally filed

Claims, Numbers

1-10 received on 11.10.2004 with letter of 08.10.2004

Drawings, Sheets

1/7-7/7 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☒ the claims, Nos.: 11-13
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 3, (4-10)-partially

   because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☒ no international search report has been established for the said claims Nos. 3, (4-10)-partially

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/ or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1,2,4-10
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1,2,4-10
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1,2,4-10
	No:	Claims	

2. Citations and explanations

see separate sheet

RE Item I

The amended claims 1-10 filed with the letter dated 08.10.2004 and received on 11.10.2004 are allowable according to Art. 34 (2)(b) PCT. The basis of the report issues on the claims as amended according to Rule 70.2 PCT.

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims: 3-completely and (4-10)-partially relate to subject-matter (.... **storing and recording** the sequence information on an information carrier, such as a computer disk) not required to be searched by this Authority , Rule 39.1(v) PCT. Therefore no International Search Report was established for claim 3 under Art. 17(2)(a) PCT.

Consequently, no opinion will be formulated with respect to novelty, inventive step and industrial applicability of the subject-matter of this claim (Rule 66.1(e), PCT).

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

The following documents (D) are referred to in this communication; the numbering will be adhered to in the rest of the procedure:

D1: WO 02 24862 A (SCHMIDT CHRISTIAN ;CYTION S A (CH)) 28 March 2002 (2002-03-28)
D2: WO 02 04943 A (SQUIBB BRISTOL MYERS CO) 17 January 2002 (2002-01-17)
D3: WO 99 64582 A (INTROGENE BV) 16 December 1999 (1999-12-16)
D4: WO 01 73000 A (MAXYGEN INC ;KEENAN ROBERT J (US); MINSHULL JEREMY (US); STEMMER W) 4 October 2001 (2001-10-04)
D5: EP 1 143 013 A (WARNER LAMBERT CO) 10 October 2001 (2001-10-10)

The document D6 was not cited in the international search report. A copy of the document is appended hereto.

D6: R. GEHWOLF ET AL.: 'FIRST PATCH, THEN CATCH: MEASURING THE ACTIVITY AND THE mRNA TRANSCRIPTS OF A PROTON PUMP IN INDIVIDUAL LILIUM POLLEN PROTOPLASTS', FEBS LETTERS, VOL. 512, PAGES 152-156, FEBRUARY 2002, (2002-02-00);

1. Novelty (Art. 33(2) PCT)

1.1 **D1** describes a multiaperture **biochip** comprises a substrate including several apertures, a recording fluid compartment and one reference fluid compartment arranged on each side of the substrate and being in contact through the apertures, a recording electrode and a reference electrode in contact with one of the compartments and adapted to measure and/or apply an electrical potential across the apertures. The biochip is useful for positioning and/or analysing samples such as cells, vesicles, cellular organelles, and fragments, derivatives, and their mixtures (claimed), for electrical and/or optical analysis, especially relating to the presence and/or activity of ion channels. The system is useful for automated and/or **high-throughput patch-clamp analysis** (e.g. for drug screening), portable biosensor analysis (e.g. for environmental analytes) and also for separation of cells and vesicles, the analysis of the sizes of cells or vesicles, the direct functional analysis of ionotropic membrane proteins, for e.g. in ligand binding studies, and/or the positioning of cells or vesicles for any suitable purpose, including optical investigations and/or microinjections. The system is useful in a method to screen libraries including compound, combinatorial chemistry, **gene and phage libraries** for the identification of candidate drugs and modulators and is well suited for probing libraries whose members are present only in small amounts.

1.2 **D6** describes a method "First patch, then catch" and measures the activity and the mRNA transcripts of a proton pump in individual Lilium pollen protoplasts. Combining the patch-clamp method with single-cell reverse transcription polymerase chain reaction (scRT-PCR) a fusicoccin-induced current reflecting the activity of the plasma membrane H+ ATPase of lily pollen protoplasts was measured and subsequently, the ATPase-encoding mRNAs were collected and amplified.

1.3 In the light of **D1**and **D6**, the subject-matter of claims 1,2 and 4-10 appears to be new under Art. 33(2) PCT.

2. Inventive step (Art. 33(3) PCT)

2.1 Methods to perform an electrophysiological measurement in the manner to isolate a single cell of interest and to isolate mRNA from that single cell of interest respectively methods to combine patch clamp technology with a method to carry out single cell PCR (polymerase chain reaction) are already well known in the prior art.

D6 describes the combination of the patch-clamp method with single-cell reverse transcription polymerase chain reaction (scRT-PCR). A fusicoccin-induced current reflecting the activity of the plasma membrane H+ ATPase of lily pollen protoplasts was measured and subsequently, the ATPase-encoding mRNAs were collected and amplified. **D6**, regarded as the closest state of the art, differs from the subject-matter that it lacks the technical feature of isolating the cell **expressing at least one heterologous DNA sequences** of interest/or genetic material therefrom; and isolating mRNA from said cell of interest identified. In the light of the prior art the problem of underlying application is the provision of an alternative method for detection and isolation of **heterologous DNA sequences**. The solution as provided by the applicant is a method for performing electrophysiological measurements compring the step of: (i) providing a substrate for making the electrophysiological measurements upon which at least one cell can be arranged; (ii) providing at plurality of cells, each cell comprising a different heterologous DNA sequence derived from a DNA library, wherein each cell expresses the heterologous DNA sequence it comprises; (iii) arranging the plurality of cells provided in step (ii) on the substrate to permit detection and/or measurement of a change (in comparison to a control cell) in the electrophysiology of each cell; and (iv) identifying at least one cell of interest which shows at least one phenotypic change, characterized in that, the method comprises the further steps of : isolating the cell of interest/or genetic material therefrom; and isolating mRNA from the cell of interest identified in step (iii).

D2 describes an **apparatus** for measuring cellular electrical conditions comprising a cell support membrane component (CSC) adapted to hold one or more cells. Said apparatus is useful for measuring cellular electrical condition such as transmembrane potential, capacitance, resistance and conductance of cells such as human embryonic kidney (HEK)-293 cells, Chinese hamster ovary cells, primary neuronal cells (preferably

hippocampus, dorsal root ganglia or superior cervical ganglia cells), skeletal muscle cells, smooth muscle cells, cardiac muscle cells, immune cells, epithelial cells, or endothelial cells. Optionally, said apparatus is useful for **measuring electrical condition** of cells comprising DNA constructs directing the expression of molecules such as ion channel proteins, ion transporters, G-proteins, G-protein receptors, protein kinases or protein phosphatases, cells expressing ion channels that are specific for ions such as sodium, potassium, calcium or chloride. Moreover said apparatus is useful in a **high throughput screening method** for detecting and assaying test agents that affect cellular electrical activity. The test agents which are assayed are e.g. G-proteins and/or G-protein receptors.

D3 describes a library of expressible nucleic acids which contains many compartments, each comprising at least one vehicle comprising at least one nucleic acid, the vehicle being capable of efficiently introducing a nucleic acid into a cell for expression. Said library is useful for determining the function of one or more nucleic acids within the library, or to screen for an expressible nucleic acid with a particular desired function. It is especially useful for high throughput screening of gene function for functional genomics applications and for screening for nucleic acids with potential therapeutic value.

D4 describes a method for controlling, a phenotype which comprises recombining or mutating a population of conjoint polynucleotide segments. Said method further comprises to encode or modulate a phenotype, to produce a library, introducing the library into a population of recipient cells or intracellular organelles and identifying a cell, organelle, or organism comprising a cell with a desired phenotype.

D5 describes the screening modulators of calcium channel (specifically calcium-release-activated channel (Icrac)) activity by: (a) contacting the modulators and a Ca channel activator with a population of Ca channel expressing cells containing a reporter construct with a reporter gene under the control of a nuclear factor of activated T cells (NFAT)-inducible promoter; and (b) determining the activity of (I) on the channel.

For a man skilled in the art it would be **not obvious** to combine the technical feature of **D6** with any one of **D2-D5** to achieve the same result as in the present application.

2.1.1 In the light of the prior art documents the subject-matter of claims 1,2 and 4-10 comprises an inventive step under Art. 33(3) PCT.

< > = < a plurality of cells, each cell comprising a different heterologous DNA sequence derived from a DNA library, wherein each >

<< >> = << , said change being a result of expression of the heterologous DNA sequence >>

CLAIMS

1. A method for performing electrophysiological measurements comprising the steps of:

(i) providing a substrate for making the electrophysiological measurements upon which at least one cell can be arranged;

(ii) providing < > ~~[at least one]~~ cell ~~[which]~~ expresses ~~[at least one]~~ the heterologous DNA sequence it comprises;

(iii) arranging the ~~[at least one]~~ plurality of cells provided in step (ii) on the substrate to permit detection and/or measurement of a change (in comparison to a control cell) in the electrophysiology of ~~[the]~~ each cell << >>, and

(iv) identifying at least one cell of interest which shows at least one phenotypic change,

characterised in that, the method comprises the further steps of:

isolating the cell of interest, and/or genetic material therefrom; and isolating mRNA from the cell of interest identified in step (iii).

2. A method as claimed in Claim 1 wherein the method further comprises the step of sequencing the genetic material.

3. A method as claimed in Claim 2 wherein the method further comprises the step of storing or recording the sequence information on an information carrier, such as a computer disk.

~~4. A method as claimed in any preceding claim wherein a plurality of cells is provided in step (ii).~~

~~5. A method as claimed in any preceding claim wherein a plurality of~~ cells is provided in step (ii), ~~with each cell containing a different heterologous DNA sequence.~~

~~6. A method as claimed in Claim 4 or 5 wherein the plurality of cells together comprises a DNA library of heterologous DNA~~

4. A method as claimed in any previous Claim wherein the DNA library is a cDNA library.

5. A method as claimed in any one of the preceding claims wherein the change in the electrophysiology of the cell is detected and/or measured by patch clamping.

6. A method as claimed in any preceding claim wherein the cell is treated with a test agent before step (iii).

7. A method as claimed in Claim 6 wherein the test agent is selected from at least one of the following: small organic molecules, small peptides, neurotransmitters, hormones and cytokines.

8. A method as claimed in any preceding claim wherein the cell is an animal cell.

9. ~~12~~. A method as claimed in any preceding claim wherein the animal cell is selected from: Human Embryonic Kidney 293 (HEK293), Chinese Hamster Ovary (CHO), COS, MDCK, NG108, NIH3T3 or T84.

10. ~~13~~. A method as claimed in any ~~one of Claims 4 to 12~~ previous claim wherein the cells are arranged at spaced-apart locations in or on the substrate.